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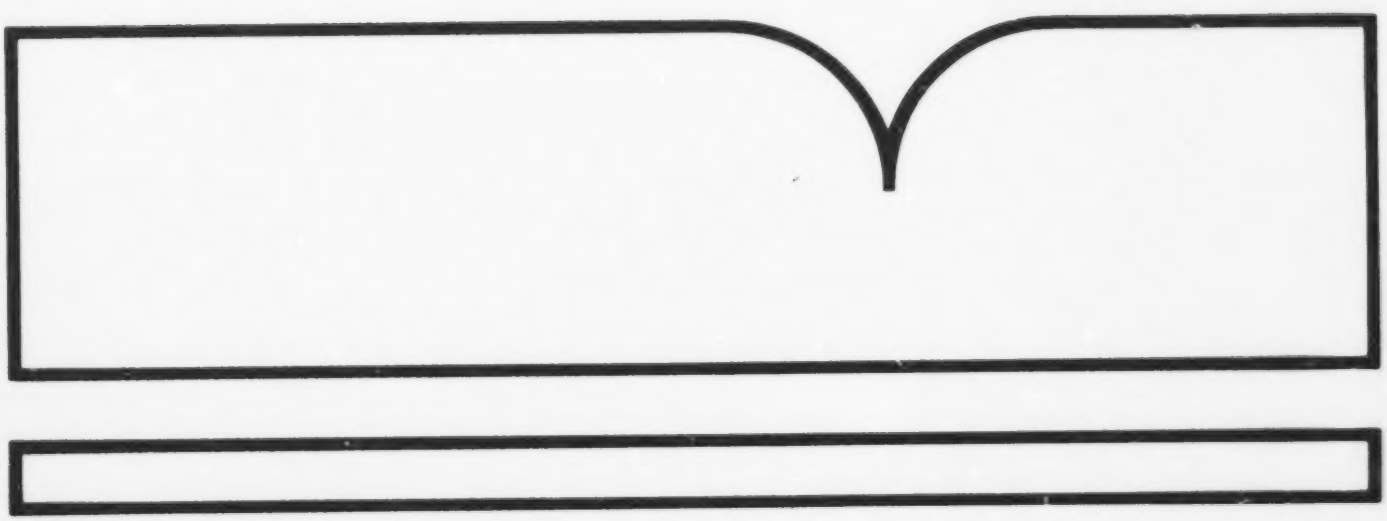

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16. ABSTRACT

Allyl alcohol was administered ip to female Fischer 344 rats at doses of 0, 3, 10 and 30 mg/kg daily for 7 days. Plasma sorbitol dehydrogenase was minimally elevated. No dose related changes were observed in hexobarbital oxidation, aniline hydroxylation, or ethylmorphine demethylation. Aldrin epoxidation was slightly elevated. Naphthol glucuronidation and glutathione-S-transferase activity with 1,2-dichloro-4-nitrobenzene were increased. Results from in vivo studies on the metabolism of lindane were in close agreement with the in vitro measurements suggesting that daily treatment for one week with allyl alcohol at doses of 3, 10 and 30 mg/kg has no significant effect on phase I pathways, a selective effect on phase II pathways and, under the conditions of this experiment, minimal hepatoxic effects in these rats.

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COMPARISON OF IN VIVO AND IN VITRO METHODS FOR ASSESSING EFFECTS OF ALLYL ALCOHOL ON THE LIVER

(Lindane; drug metabolism; liver)

BRUCE A. TRELA[a], GARY P. CARLSON[a*], ROBERT W. CHADWICK[b] and M. FRANK COPELAND[b]

[a]*Department of Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907 and* [b]*Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711 (U.S.A.)*

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SUMMARY

Allyl alcohol was administered intraperitoneally (i.p.) to female Fischer 344 rats at doses of 0, 3, 10 and 30 mg/kg daily for 7 days. Plasma sorbitol dehydrogenase was minimally elevated. No dose-related changes were observed in hexobarbital oxidation, aniline hydroxylation, or ethylmorphine demethylation. Aldrin epoxidation was slightly elevated. Naphthol glucuronidation and glutathione-*S*-transferase activity with 1,2-dichloro-4-nitrobenzene were increased. Results from in vivo studies on the metabolism of lindane were in close agreement with the in vitro measurements suggesting that daily treatment for one week with allyl alcohol at doses of 3, 10 and 30 mg/kg has no significant effect on phase I pathways, has a selective effect on phase II pathways and , under the conditions of this experiment, has minimal hepatotoxic effects in these rats.

INTRODUCTION

Allyl alcohol has been widely used as a synthetic intermediate in industry. Its hepatotoxicity is primarily seen in the periportal region of the liver, probably due to its metabolism in that area by alcohol dehydrogenase [1,2]. It has been shown to inhibit microsomal reactions including ethylmorphine demethylation [3], aniline hydroxylation [4] and glucose-6-phosphatase activity [5] following single doses. Conjugation reactions have been found to be resistant to inhibition [3].

*To whom correspondence should be addressed.

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The purpose of the present study was 2-fold. One was to examine the effect of repeated doses of allyl alcohol on the liver including indicators of hepatotoxicity, mixed function oxidase reactions and conjugative enzymes. The second was to compare the sensitivity, both qualitative and quantitive, of the usual in vitro methods employed in such studies with the in vivo system of Chadwick et al. [6,7] in which lindane serves as a model substrate for measuring effects on both phase I and phase II reactions.

MATERIALS AND METHODS

In both the studies made on in vitro measurements and those on lindane metabolism in vivo, young female Fischer 344 rats (Charles River Breeding Laboratories, Kingston, NY) were used. They were housed singly in stainless steel cages with free access to food (Purina Lab Chow, Ralston Purina, St. Louis, MO) and water. Lights were on a 12:12 h light:dark cycle with the temperature maintained at 21°C. Bedding was Bed-O-Cobs (Andersons Cob Division, Delphi, IN).

After an adaptation period of one week, allyl alcohol (Fisher Scientific, Fair Lawn, NJ) was administered i.p. to groups of 6 rats at doses of 3, 10 and 30 mg/kg daily for 7 days. The dosing volume was 1% of body weight. 24 h After the last dose, blood was obtained from the orbital sinus for the measurement of plasma sorbitol dehydrogenase [8]. Whole liver homogenates were used for determination of glucose-6-phosphatase [9] and the glucuronidation of 1-naphthol and chloramphenicol [10]. Microsomal and cytosolic fractions were prepared by differential centrifugation. Ethylmorphine demethylation was measured by the method of La Du et al. [11], aniline hydroxylation by the method of Imai et al. [12], hexobarbital oxidation by the method of Kupfer and Rosenfeld [13,14] and aldrin epoxidation by the method of Wolff et al. [15]. Glutathione-*S*-transferase activity in the cytosolic fraction was determined using the aryl substrate 1,2-dichloro-4-nitrobenzene and the epoxide substrate 1,2-epoxy-3-(*p*-nitrophenoxy)propane [16]. Proteins were quantified using the method of Lowry et al. [17].

On day 8 at the laboratory, all rats were dosed subcutaneously (s.c.) with 20 mg lindane (containing 3.5 μCi [U-^{14}C]lindane)/kg. [U-^{14}C]lindane, with a specific activity of 54 mCi/mmol and a radiochemical purity of 98%, was obtained from Amersham (Arlington Heights, IL). Following treatment on day 8 the animals were transferred to animal containment chambers (Plas-Labs, Lansing, MI) and urine, feces and expired air were collected for 24 h.

A 10-ml urine aliquot was made acid (pH 2.0) with HCl and extracted twice with equal volumes of isooctane for free chlorophenols, alcohols and unaltered lindane. The residual urine was hydrolyzed with β-glucuronidase (Sigma Chemical Co., St. Louis, MO) by a previously reported procedure [18] and, after acidification, was extracted twice with equal volumes of isooctane to obtain the chlorophenol and alcohol glucuronides. Residual urine from this extraction was hydrolyzed with

arylsulfatase (Sigma, St. Louis, MO) by a previously reported method [18] and after acidification was extracted twice with equal volumes of isooctane to obtain the chlorophenol and alcohol sulfates. Residual urine from this extraction was hydrolyzed and acylated as previously described by Allsup and Walsh [19] and extracted twice with isooctane for determination of the mercapturic acid metabolites of lindane. Urine extracts were analyzed for lindane metabolites on a Tracor Model MT-220 gas chromatograph equipped with ^{63}Ni electroncapture detector and linearizer. The column consisted of a 183 × 0.63 cm O.D. glass U-tube packed with 5% EGS + 1% H_3PO_4 on 90/100-mesh Anakrom A (Analabs, North Haven, CT). The column was maintained isothermally at 170°C. Inlet and detector temperatures were 245°C and 330°C, respectively. The 5% methane/argon carrier gas flow rate was regulated at 55 cm^3/min. Radioactivity was analyzed in a Packard Tri-Carb Model 3380 liquid scintillation spectrometer using a quench correction curve and a scintillation solution containing 500 ml toluene, 500 ml cellosolve, 5.0 g 2,5-diphenyloxazole (PPO) and 50 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP).

Duncan's Multiple Range Test [20] was used as an aid in the interpretation of the data from this study. Treatments were considered significantly different at $P < 0.05$.

RESULTS

Allyl alcohol was not significantly toxic under these conditions. Glucose-6-phosphatase activity was not inhibited and was actually slightly increased at the high dose (Table I). Only a slight increase in plasma sorbitol dehydrogenase was observed at the high dose. Allyl alcohol had little effect on the microsomal mixed function oxidase enzymes. Ethylmorphine demethylation was not inhibited at any dose (Table II). Although hexobarbital oxidation and aniline hydroxylation were increased at the low dose, there were no dose-related changes. Aldrin epoxidation was somewhat elevated at the two higher doses.

Allyl alcohol increased glucuronyltransferase activity with 1-naphthol but not with chloramphenicol thus demonstrating substrate selectivity (Table III). Selectivi-

TABLE I

EFFECT OF ALLYL ALCOHOL ON INDICATORS OF HEPATOTOXICITY

Treatment[a]	Glucose-6-phosphatase (μmol/g liver/min)	Sorbitol dehydrogenase (μmol fructose/ml serum/h)
Control	27.6 ± 2.6[b]	1.36 ± 0.13[b]
Allyl-OH, 3 mg/kg	26.8 ± 3.5[b]	1.34 ± 0.05[b]
Allyl-OH, 10 mg/kg	25.5 ± 1.4[b]	1.32 ± 0.03[b]
Allyl-OH, 30 mg/kg	35.7 ± 1.5[c]	1.89 ± 0.27[c]

[a]Allyl alcohol was administered i.p. to groups of 6 rats daily for 7 days. Controls received distilled water (vehicle). Rats were killed 24 h after the last dose.

[b,c]Values are means ± SEM. Values within the same column with different superscripts are significantly different ($P \leq 0.05$).

TABLE II

EFFECT OF ALLYL ALCOHOL ON MICROSOMAL XENOBIOTIC METABOLISM

Treatment[a]	Ethylmorphine demethylation (nmol HCHO/mg protein/min)	Hexobarbital oxidation (nmol/mg protein/min)	Aniline hydroxylation (nmol/mg protein/min)	Aldrin epoxidation (nmol/mg protein/min)
Control	4.30 ± 0.08[b,c]	1.87 ± 0.20[b]	0.62 ± 0.02[b]	0.090 ± 0.013[b]
Allyl-OH, 3 mg/kg	4.63 ± 0.13[c]	2.42 ± 0.19[c]	0.74 ± 0.04[c]	0.114 ± 0.007[b,c]
Allyl-OH, 10 mg/kg	4.18 ± 0.20[b]	1.81 ± 0.17[b]	0.60 ± 0.04[b]	0.184 ± 0.035[c]
Allyl-OH, 30 mg/kg	4.49 ± 0.04[b,c]	1.73 ± 0.04[b]	0.57 ± 0.03[b]	0.166 ± 0.016[c,d]

[a]Allyl alcohol was administered i.p. to groups of 6 rats daily for 7 days. Controls received distilled water(vehicle). Rats were killed 24 h after the last dose.

[b,c,d]Values are means ± SEM. Values within the same column with different superscripts are significantly different ($P \leq 0.05$).

ty was also shown for the glutathione-*S*-transferases, where conjugation with 1,2-dichloro-4-nitrobenzene but not with 1,2-epoxy-3-(*p*-nitrophenoxy)propane was increased at the 30 mg/kg dose level.

The in vivo studies on lindane metabolism supported the in vitro measurements in that there were few changes in the allyl alcohol-treated groups. The total percentage of [^{14}C]lindane administered to control rats and recovered as urinary metabolites within 24 h was 11.6. The values for the 3 mg/kg (16.0%), 10 mg/kg (16.7%) and 30 mg/kg (18.2%) treatment groups were not statistically different ($P > 0.05$) from the controls. Glucuronide conjugates were increased at all 3 dose levels (Fig. 1), but GLC analysis of the lindane metabolites indicated that only excre-

TABLE III

EFFECT OF ALLYL ALCOHOL ON CONJUGATION REACTIONS

Treatment[a]	Glutathione-*S*-transferase		Glucuronyltransferase	
	1,2-Dichloro-4-nitrobenzene (nmol/mg protein/min)	1,2-Epoxy-3-(*p*-nitrophenoxy)propane (nmol/mg protein/min)	1-Naphthol (nmol/g liver/min)	Chloramphenicol (nmol/g liver/min)
Control	29.9 ± 1.2[b]	46.8 ± 2.0[b]	1044 ± 97[b]	27.0 ± 1.0[b]
Allyl-OH, 3 mg/kg	24.7 ± 1.1[b]	36.5 ± 2.4[c]	1026 ± 60[b]	27.9 ± 1.0[b]
Allyl-OH, 10 mg/kg	27.6 ± 1.5[b]	39.4 ± 3.0[b,c]	1511 ± 101[c]	29.7 ± 1.6[b]
Allyl-OH, 30 mg/kg	40.6 ± 2.9[c]	45.1 ± 4.0[b,c]	1354 ± 79[c]	27.4 ± 1.1[b]

[a]Allyl alcohol was administered i.p. to groups of 6 rats daily for 7 days. Controls received distilled water (vehicle). Rats were killed 24 h after the last dose.

[b,c]Values are means ± SEM. Values within the same column with different superscripts are significantly different ($P \leq 0.05$).

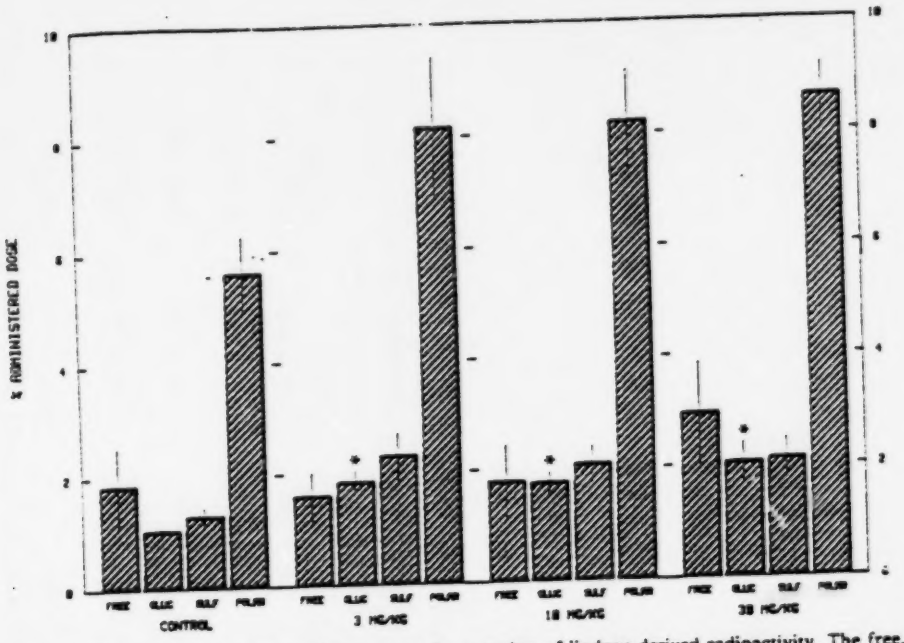

Fig. 1. Effect of allyl alcohol pretreatment on the excretion of lindane-derived radioactivity. The free, glucuronide, sulfate, and polar (mercapturic acid) metabolites were extracted and analyzed as described in METHODS. Each bar is the mean of 6 rats. The vertical lines indicate the SE. *, Significant difference from control.

tion of the alcohol glucuronides was significantly elevated by the allyl alcohol pretreatment (Table IV). Similarly when the total sulfate conjugates were assayed by determining the radioactivity excreted in this fraction, there were no treatment differences (Fig. 1), but GLC analysis (Table IV) indicated that urinary alcohol sulfates were elevated while chlorophenols were not. Only excretion of the combined 2,4-and 2,5-dichlorophenylmercapturic acid metabolites showed a dose-related increase in the allyl alcohol-treated rats. The effect of allyl alcohol pretreatment on these phase II pathways supports the selectivity observed in the in vitro measurements.

DISCUSSION

The hepatotoxic effects of allyl alcohol have been demonstrated by a number of investigators [1, 2, 21]. However in the present study, allyl alcohol was not highly hepatotoxic. This may be related to either repeated treatment, the use of females or the age of the animals. For example, it has been reported that the daily administration of bromobenzene, usually considered as a hepatotoxin, for 4 weeks, resulted in increases in both microsomal mixed-function oxidase and conjugative ac-

TABLE IV
EFFECT OF PRETREATMENT WITH ALLYL ALCOHOL ON PHASE II REACTIONS IN VIVO

Treatment	Glucuronidation		Sulfate conjugation		Glutathione conjugation	
	Alcohol glucuronides[a]	Chlorophenol glucuronides[b]	Alcohol sulfates[c]	Chlorophenol sulfates[d]	Mercapturic Acids	
					Total[e]	24/25-DCMA[f]
1. Control	0.072 ± 0.008	0.38 ± 0.05	0.19 ± 0.04	0.83 ± 0.12	5.72 ± 0.36	0.09 ± 0.13
2. 3 mg/kg	0.090 ± 0.012	0.85 ± 0.15	0.19 ± 0.04	1.52 ± 0.13	8.59 ± 0.67	0.16 ± 0.03
3. 10 mg/kg	0.140 ± 0.024	0.67 ± 0.11	0.23 ± 0.07	1.16 ± 0.25	8.46 ± 1.69	0.18 ± 0.03
4. 30 mg/kg	0.161 ± 0.03	0.66 ± 0.16	0.41 ± 0.07	1.14 ± 0.13	8.13 ± 0.82	0.20 ± 0.02
Treatment Comparison	4,3 > 1	N.S.	4 > All	N.S.	N.S.	All > 1

Values are means ± SEM from 6 rats. Comparisons were considered significantly different at $P<0.05$ by Duncan's Multiple Range Test. N.S., not significantly different at $P<0.05$.

[a] % Administered lindane excreted as alcohol glucuronides per 24 h.

[b] % Administered lindane excreted as chlorophenol glucuronides per 24 h.

[c] % Administered lindane excreted as alcohol sulfates per 24 h.

[d] % Administered lindane excreted as chlorophenol sulfates per 24 h.

[e] % Administered lindane excreted as mercapturic acids per 24 h.

[f] % Administered lindane excreted as combined 2,4- and 2,5-dichlorophenylmercapturic acid per 24 h.

tivities [22]. When female rats were repeatedly pretreated with subtoxic doses of the hepatotoxin chlorobenzene, this chemical accelerated its own metabolism and reduced chlorobenzene-induced centrilobular hepatocellular necrosis [23]. Furthermore, Rikans [24] has shown that older rats are more susceptible to the hepatotoxic effects of allyl alcohol than are younger rats. However, attempts to raise the dose level to 60 mg/kg resulted in the death of 3 of 5 rats (data not shown). Although previous studies have indicated decreases in mixed function oxidases following allyl alcohol administration [3,4], minimal effects were observed in the present investigation. Increases in the glucose-6-phosphatase, UDP-glucuronyltransferase, and aldrin epoxidation may reflect an effect on the membranes of the endoplasmic reticulum.

A comparison of the in vitro and in vivo data showed good correlations both qualitatively and quantitatively. In vivo no difference was observed in free or total ^{14}C-lindane metabolites excreted in the urine. Alcohol glucuronides were preferentially increased in agreement with the selective increase in the glucuronidation of 1-naphthol in vitro. Moreover, the specific increase in the urinary content of the combined 2,4- and 2,5-dichlorophenylmercapturic acid metabolites of lindane after allyl alcohol pretreatment was consistent with the selective effect of this pretreatment on the in vitro glutathione-*S*-transferase activity. Thus after allyl alcohol pretreatment, glutathione-*S*-transferase activity was significantly increased when 1,2-dichloro-4-nitrobenzene but not when 1,2-epoxy-3-(*p*-nitrophenoxy) propane was employed as the substrate. In general, it is concluded that the in vitro and in vivo data support the use of in vivo measurements such as lindane metabolism for studying the integrated effects of toxicants such as allyl alcohol.

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REFERENCES

1 K.R. Rees and M.J. Tarlow, The hepatotoxic action of allyl formate, Biochem. J., 104 (1967) 757–761.

2 J.M. Patel, W.P. Gordon, S.D. Nelson and K.C. Leibman, Comparison of hepatic biotransformation and toxicity of allyl alcohol and [1,1-$^{3}H_2$]allyl alcohol in rats, Drug Metab. Dispos., 11 (1983) 164–166.

3 Z. Gregus, J.B. Watkins, T.N. Thompson and C.D. Klaassen, Resistance of some phase II

biotransformation pathways to hepatotoxins, J. Pharmacol. Exp. Ther., 222 (1982) 471–479.
4 R. James, P. Desmond, A. Kupfer, S. Schenker and R.A. Branch, The differential localization of various drug metabolizing systems within the rat liver lobule as determined by the hepatotoxins allyl alcohol, carbon tetrachloride and bromobenzene, J. Pharmacol. Exp. Ther., 217 (1981) 127–132.
5 G. Feuer, L. Golberg and J.R. LePelley, Liver response tests, I. Exploratory studies on glucose-6-phosphatase and other liver enzymes, Food Cosmet. Toxicol., 3 (1965) 235–249.
6 R.W. Chadwick, C.J. Chadwick, J.J. Freal and C.C. Bryden, Comparative enzyme induction and lindane metabolism in rats pretreated with various organochlorine pesticides, Xenobiotica, 7 (1977) 235–246.
7 R.W. Chadwick, M.F. Copeland, M.L. Mole, S. Nesnow and N. Cooke, Comparative effect of pretreatment with phenobarbital, Aroclor 1254 and β-naphthoflavone on the metabolism of lindane, Pest. Biochem. Physiol. 15 (1981) 120–136.
8 U. Gerlach, Sorbitol dehydrogenase, in H.U. Bergmeyer (Ed.), Methods of Enzymatic Analysis, Academic Press, New York, 1963, pp. 761–764.
9 A.E. Harper, Glucose-6-phosphatase, in H.U. Bergmeyer (Ed.), Methods of Enzymatic Analysis, Academic Press, New York, 1963, pp. 788–792.
10 K.W. Bock, G. Brunner, H. Hoensch, E. Huber and D. Josting, Determination of microsomal UDP-glucuronyltransferase in needle-biopsy specimens of human liver, Eur. J. Clin. Pharmacol., 14 (1978) 367–373.
11 B.N. LaDu, H.G. Mandel and E.L. Way (Eds.), Fundamentals of Drug Metabolism and Distribution, Williams and Wilkins, Baltimore, 1971, pp. 562–566.
12 Y. Imai, A. Ito and R. Sato, Evidence for biochemically different types of vesicles in hepatic microsomal fraction, J. Biochem. (Tokyo), 60 (1966) 417–428.
13 D. Kupfer and J. Rosenfeld, A sensitive radioactive assay for hexobarbital hydroxylase in hepatic microsomes, Drug Metab. Dispos., 1 (1973) 760–765.
14 D. Kupfer, Correspondence: Assay of hexobarbital hydroxylase, Drug Metab. Dispos., 6 (1978) 610.
15 T. Wolff, E. Deml, and H. Wanders, Aldrin epoxidation, a highly sensitive indicator specific for cytochrome P450-dependent mono-oxygenase activities, Drug. Metab. Dispos., 7 (1979) 301–305.
16 W.H. Habig, M.J. Pabst and W.B. Jakoby, Glutathione-*S*-transferases: the first step in mercapturic acid formation, J. Biol. Chem., 249 (1974) 7130–7139.
17 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193 (1951) 265–275.
18 D.E. Rickert and R.M. Long, Metabolism and excretion of 2,4-dinitrotoluene in male and female Fischer 344 rats after different doses, Drug Metab. Dispos. 9 (1981) 226–232.
19 T. Allsup and D. Walsh, Gas chromatographic analysis of chlorophenylmercapturic acid lindane metabolites, J. Chromatogr. 236 (1982) 421–428.
20 D.B. Duncan, Multiple range and multiple F tests, Biometrics, 11 (1955) 1–42.
21 S.K. Hanson and M.W. Anders, The effect of diethyl maleate treatment, fasting and time of administration on allyl alcohol hepatotoxicity, Toxicol. Lett., 1 (1978) 301–305.
22 S. Chakrabarti, Increased activity of certain hepatic drug-metabolizing enzymes due to subchronic exposure to bromobenzene, Toxicol. Lett., 20 (1984) 79–83.
23 R.W. Chadwick, T.M. Scotti, M.F. Copeland, M.L. Mole, R. Froehlich, N. Cooke and W.K. McElroy, Antagonism of chlorobenzene-induced hepatotoxicity by lindane, Pest. Biochem. Physiol. 21 (1984) 148–161.
24 L.E. Rikans, Influence of aging on the susceptibility of rats to hepatotoxic injury, Toxicol. Appl. Pharmacol., 73 (1984) 243–249.